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Utility of Toxicity Tests to Measure Effects of Substances on Marine Organisms

David J. Hansen

Introduction

Toxicity tests using single species, microcosms and communities are the foundation for laboratory evaluations of marine (estuarine and oceanic) pollution effects. Hazard evaluation techniques use toxicity tests to predict the need for environmental concern on new and old chemicals. Interpretation of the results of field investigations can be aided through the use of laboratory tests, where variables can be controlled and effects quantified. While not a panacea, laboratory toxicity tests are generally accepted by industry, university and government scientists as one of the techniques useful in measuring the effects of marine pollution.

Discussions in this paper will emphasize acute, early life-stage, life cycle and community toxicity tests with marine (estuarine or oceanic) species as conducted at the U.S. EPA Environmental Research Laboratory at Gulf Breeze, Florida. These tests are emphasized because of their importance in the hazard evaluation process, as discussed in workshop proceedings by Cairns et al. (1978) and Dickson et al. (1979). Individual papers published in the proceedings of these two workshops detail hazard evaluation techniques used by the American Institute of Biological Science, American Society for Testing and Materials, Monsanto Company, U.S. Environmental Protection Agency and by Japanese and French scientists. In addition, testing requirements for effects assessments have been recently identified for development of water quality criteria by the U.S. Environmental Protection Agency (1980a) and the U.S. Army (Pearson and Glennon, 1979). Although the application of each of these

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16. ABSTRACT

Toxicity tests using single species, microcosms, and communities of test species are described for laboratory evaluations of marine (estuarine and oceanic) pollution effects. The design of acute, early life-stage, life-cycle, and community toxicity tests is discussed. Uses of test data, sources of variability, species amenable to testing, and availability of standardized methods are defined.

17. KEY WORDS AND DOCUMENT ANALYSIS		
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hazard evaluation procedures differs considerably, the principal effects testing requirements include those discussed herein. These laboratory effects tests have been pivotal in the majority of these evaluations of hazard because these evaluation processes occur before the chemical impacts the environment and are used only rarely to measure the extent of impact in an already contaminated environment.

Acute, early life-stage, life-cycle and community toxicity tests will be discussed separately. As a part of each discussion, the design of the test, uses of the data, sources of variability, species amenable to testing, availability of standardized methods and the relationships to data from other test types will be discussed where appropriate. Much of the summary information was assembled as a result of developing water quality criteria for the consent decree chemicals or from the data used in the criteria documents (U.S. EPA, 1980a,b).

ACUTE TOXICITY TESTS

Results from acute toxicity tests are extremely useful, but have probably been more maligned than results from any other type of toxicity test. Because acute toxicity tests are completed in a short time, they are relatively inexpensive and usually provide the first data on the toxicity of individual chemicals or complex mixtures. Standardized methods for conducting acute toxicity tests are available (American Society for Testing and Materials, 1980; APHA-AWWA-WPCF, 1980) and information useful in design or interpretation of results are also available (Sprague, 1969; 1970; 1971; Buikema et al., 1982), but unfortunately there are no standards for using or evaluating the results in environmental situations where concentrations and bioavailability change over time; herein lies the problem. Acute toxicity test data can be particularly useful in the first stages of product development. Data from acute toxicity

tests are used to select exposure concentrations for chronic toxicity, bioconcentration and other tests used in the evaluation process. The comparative acute sensitivities of species and life-stages of marine organisms to a particular chemical can aid in identifying populations at risk.

Acute toxicity tests can be used to measure the bioavailability/toxicity of complex wastes. Chemical fractionation of wastes followed by toxicity tests can aid in identification of the toxic fraction (Walsh and Garnas, manuscript). Conversely, data from tests of individual chemicals rarely can be used to predict the overall effect of complex wastes or contaminated sediments that contain mixtures of a great many chemicals.

The most appropriate use of acute toxicity tests is to measure the toxicity of substances or sensitivity of species to substances, not to predict environmental hazard directly. In some instances, however, results from acute toxicity tests may be directly applicable to situations in which environmental and acutely lethal concentrations and availability are similar.

Relatively few marine species have been used in acute toxicity tests and, of those species that have been tested, most have been fishes or arthropods. For example, the 64 water quality criteria documents concerning the consent decree chemicals, which include many of the intensively studied metals and pesticides, report acute toxicity test data on only 76 species of marine animals (Table 1). Arthropods and fishes account for 75 percent of the animal species tested. Only 10 percent of the species used were tested on more than 10 of the chemicals. Eleven species of mollusks were tested; four were gastropods, accounting for only 11 percent of the data on mollusks. Thirty percent of the 76 animal species were tested with only one chemical. The lack of data on comparative sensitivities of species of phytoplankon and macro-algae is even greater for consent-decree chemicals than for tests on animals.

Because most of the acute toxicity test data are concentrated on a small number of species in a few phyla, minimal knowledge of the relative sensitivities of untested or little tested taxa may inhibit understanding of their relative sensitivities.

Variation in the relative acute sensitivity of animal species to chemicals is considerable. For those pesticides or metals for which marine water quality criteria are available, the ratio of LC50 values for the species least sensitive to the most sensitive to a given chemical ranged from 20 to 18,000, averaging 600 for metals and 3,800 for pesticides (Table 2). The variation in sensitivity among species is far greater than the variation that occurs within a species as a function of the acute toxicity test method. The degree of variation in acute sensitivity of species perhaps is a toxicological property of individual chemicals that is important in determining the adequacy of data for decision making.

A second factor important to the adequacy of data is the need for information on the sensitivity of a variety of phylogenetic groups to identify the most sensitive taxa and to optimize testing. Certain types of organisms such as arthropods, including penaeid shrimp, mysids and copepods, have been generally recognized as extremely sensitive. Examination of water quality criteria documents demonstrates that these taxa frequently are extremely sensitive, but can also be relatively insensitive (Table 2). Therefore, assessments of acute hazard or specific substances may require a reasonable spread in the kinds of species tested to identify phylogenetic groups that are most sensitive.

Measurement of the variability of data from standardized acute toxicity tests is important if results are to be used in decision-making. Schimmel (1981) reports on an interlaboratory comparison of the results of acute

toxicity tests that used the method described in "Standard practice for conducting basic acute toxicity tests with fishes, macroinvertebrates and amphibians; Draft No. 6 (ASTM, 1977). The six participating laboratories conducted static 96-hour toxicity tests on sheepshead minnows (Cyprinodon variegatus), the bay mysid (Mysidopsis bahia) and the copepod (Acartia tonsa), using the pesticide, endosulfan and silver ($AgNO_3$). Flow-through tests were also conducted on the first two organisms and these chemicals. An analysis of the results reported by Schimmel (1981) indicated that, except for the copepod tests where biological problems existed, the variability of the results was acceptable (Table 3). The coefficient of variation for the fish and mysid data ranged from 0.27 to 0.62 (average 0.43), depending on the test type or chemical. Results were similar; even though laboratories had a varied experience with the test and the test species, laboratories failed to follow completely all requirements of the test, some fish were feral and some lab raised, and the source of dilution water and organisms varied, being from estuaries along the Gulf of Mexico to New England.

CHRONIC TOXICITY TESTS

Life-cycle and early life-stage toxicity tests

Entire life-cycle toxicity tests begin with fish embryos (Figure 1) or newly released invertebrate larvae, and partial life-cycle toxicity tests begin with juveniles; both continue through development to mature individuals and production of progeny. Test duration ranges from a few weeks for invertebrates to 5 to 8 months for fish. Toxicologists who wish to conduct life-cycle tests with marine organisms must use procedures in published literature because standard methods are not avaiable. Use of procedures in APHA-AWWA-WPCF (1980) should not generally be attempted without consulting original references.

The primary endpoints include determination of effects on survival, growth and reproduction. Secondarily, pathological, behavioral and physiological effects and bioconcentration of the chemical may be measured. The lowest concentration that causes statistically significant effects on the primary endpoints and the highest concentration at which statistical effects were not detected are used to set limits termed the maximum acceptable toxicant concentration (MATC). The primary effect endpoints can also be used with population dynamics models to measure the effect of substances on the intrinsic rate of population increase (Allen and Daniels, 1980; Daniels and Allan, 1981; Gentile et al., In press a). The MATC or intrinsic rate of increase is used to estimate the "safe" concentration for that species and chemical.

Only a few marine species have been successfully used in life-cycle toxicity tests, and only two have been tested with enough chemicals to provide comparative toxicological information (Table 4). Life-cycle tests with the bay mysid (Nimmo et al. 1978) and the sheepshead minnow (Hansen et al., 1978) probably provide over 75 percent of all available data on the life-cycle effects of substances on marine organisms. More than thirty tests have been conducted on these two species. In contrast, only one or two life-cycle tests have been completed with species such as the mollusk, Crepidula fornicata (Nelson et al., In manuscript), the copepods, Acartia tonsa (Ward et al., 1979) and Eurytemora affinis (Daniels and Alan, 1981), the grass shrimp, Palaemontes pugio (Tyler-Schroeder, 1979) and the mysid, Mysidopsis bigelowi (Gentile, In press b).

Early life stage toxicity tests with marine fish begin with embryos and continue through embryonic development, hatching and growth to the juvenile

stage. Duration is typically 4 to 8 weeks, depending upon species or the need to extend the exposure because effects continue beyond the usual test duration. Consensus early life-stage toxicity test methods for marine and freshwater fishes are being developed (ASTM, 1982). Few marine species have been used in early life-stage toxicity tests; these include the sheepshead minnow, Cyprinodon variegatus (Goodman et al., 1978), the silversides, Menidia peninsulae (Goodman et al., In manuscript) and M. menidia, the California grunion, Leuresthes tenius and the Gulf toadfish, Opsanus beta (Table 4). Sheepshead minnow is the only species that has been with tested with several chemicals. The primary endpoints of early life-stage tests include determination of effects on embryonic development, hatching success, survival of hatched fish and growth. Secondarily, pathological, behavioral and physiological effects and bioconcentration of the chemical can be measured.

Results from early life-stage toxicity tests with freshwater fishes have demonstrated that MATC's from life-cycle toxicity tests can usually be estimated within a factor of two, using MATC's derived from survival or growth data from early life-stage tests (McKim, 1977; Macek and Sleight, 1977). The only data base for marine fishes is that from life-cycle and early life-stage tests with sheepshead minnows. Slightly more that 70 percent of the life-cycle MATC's were estimated within a factor of two from estimated MATC's derived from early life-stage tests within life-cycle tests on this species (Table 5). This relationship between early life-stage and life-cycle MATC's is not as clear if data from early life-stage tests that began with embryos from unexposed parents are used. Only seven chemicals have been so tested; four (57%) predicted life cycle MATC's poorly. Overall, however, the early life-stages of both marine and freshwater fishes are as sensitive, or more sensitive, than later life-stages including reproduction. Therefore, early

life-stage tests acceptably predict life-cycle MATC's.

Examination of the frequency of occurrence of survival, growth or reproduction as the most sensitive endpoint in chronic toxicity tests with marine fish and invertebrates (Table 6) is especially interesting relative to the conclusions reached in the comparisons made above. For early life-stage toxicity tests, survival and growth were equally sensitive; in some instances, both were reduced at the lowest effect concentration. In life-cycle toxicity tests with sheepshead minnows, grass shrimp and bay mysids, reproduction (the number of embryos or young released per female, or the percentage fertility) was usually the most sensitive endpoint. Reproductive impairment was the most sensitive endpoint in sheepshead minnow life-cycle tests and this may seem to contradict the conclusion that early life-stage tests can be useful in estimating life-cycle test results. However, there is no contradiction, because reproductive effects frequently occur at the same concentration, or at a concentration within a factor of two of the concentration that affected survival or growth of early life-stages. In those instances where early life-stage tests are poor predictors of life-cycle MATC's because the reproductive process was particularly sensitive, no clues may be evident in the early life-stage test. However, signs of poisoning, including physiological or behavioral effects, at concentrations that do not affect survival or growth may indicate that reproductive effects could occur and that life cycle tests may be desirable. This is particularly true when environmental concentrations are close to those that affect the early life-stages of fishes.

Chronic toxicity tests provide data useful in predicting "safe" concentrations. Mount and Stephan (1967), Eaton (1973) and Buikema et al. (1982) discuss the use and limitations of empirically derived application

factors, the ratio of the MATC to the LC50, in predicting "safe" concentrations when only acute toxicity data are available for other more sensitive species. Mount (1977) summarized data on freshwater fishes to identify whether the application factor for any one chemical was similar for different fishes tested in life-cycle tests. For the ten chemicals with tests on from two to six freshwater fishes, the spread of application factors across species was reasonably narrow, from 2.4 to 5.8, but for the remaining five chemicals, the spread was large, from 13 to 206. Therefore, he concluded that the spread in their numerical values was unacceptable and that a better method than the application factor concept was needed to predict concentrations that will not affect survival, growth or reproduction.

An examination of this concept using data from life-cycle tests with sheepshead minnows and data on freshwater fishes and the same chemicals may be useful. Of the eleven pesticides tested in life-cycle exposures using sheepshead minnows, six have also been tested with from one to four species of freshwater fish (Table 7). These data support the application factor concept because, within a factor of four, the application factors are similar between species for the same chemical; the effect of diazinon on flagfish is the lone exception. Therefore, extrapolation of application factors that have been experimentally determined for a particular chemical to other fishes and that chemical may be justified; particularly in instances where data on more than one species supports their applicability. However, because application factors for freshwater fishes are quite variable or may be completely inappropriate and data on the chronic sensitivity of additional marine fishes are unavailable, additional research is needed to identify new techniques for predicting safe concentrations in aquatic environments.

The interlaboratory variability of chronic toxicity tests, including the life-cycle toxicity test using Mysidopsis bahia (Nimmo et al, 1978) and the early life-stage toxicity test using Cyprinodon variegatus (ASTM, 1982), is being determined. Six laboratories conducted the mysid test with both silver ($AgNO_3$) and endosulfan (McKenney, 1982). Acceptable tests were completed by only three laboratories with silver and four laboratories with endosulfan. Tests were judged unacceptable if critical requirements in the method were violated. The average of the geometric means of the MATC limits of the three tests using silver was 42 µg/ℓ; coefficient of variation, 0.57. The average of the geometric means of the MATC limits of the four tests with endosulfan was 0.34 µg/ℓ, coefficient of variation, 0.38. The average of the two coefficient of variations was 0.48, a value similar to the overall coefficient of variation (0.43) of acute toxicity tests. Seven laboratories have completed duplicate early life-stage toxicity tests with C. variegatus, using endosulfan and pentachlorobenzene. Although final analyses of data and test acceptability have not been completed, variability appears similar to that seen in the acute toxicity tests and the mysid life-cycle toxicity tests.

Community Toxicity Test

A common criticism of toxicity tests in which species are exposed separately is that extrapolation of results to protect communities which consist of a large number of interacting species is not justified. In an attempt to examine these interactions, a toxicity test has been developed at the Gulf Breeze Laboratory. The effects of substances on development and structure of estuarine communities in sand-filled aquaria can be assessed by comparing numbers, species and phyla of benthic animals that grow from planktonic larvae entering replicated control aquaria and aquaria contaminated with up to three concentrations of a substance (Hansen and Tagatz, 1980). This

approach has advantages over single-species toxicity tests, microcosms and field studies in obtaining insight into community impacts. In these other tests, an adequate number of species may not have been tested, methods used may be inappropriate, species interactions may be absent, species composition may be artificial and field studies usually lack adequate controls and replication.

The apparatuses used in these experiments were variations of two primary designs. In the first type, raw seawater with its natural component of plankton was pumped into head boxes and then flowed by gravity into four apparatuses; one served as a control and three received different concentrations of the substance to be tested. Each apparatus consisted of 6, 8 or 10 replicate aquaria (Hansen, 1974; Tagatz and Ivey, 1981; Tagatz et al., 1982). The surface area of aquaria averaged approximately 400 cm^2; each contained 5 cm of sand as a substrate for colonization covered by 3 cm of water. Flow to each aquarium was maintained at 200 ml/min. In these tests, the larvae, settling stages, juveniles and adults were exposed in the laboratory to the test substance for from 2 to 4 months. The second type, used in field studies consisted of individual aquaria 32 x 32 x 6 cm deep which were filled with sand and placed in the estuary. After about eight weeks of colonization, the 32 aquaria were removed and placed in the laboratory for one to two weeks, eight received seawater only and served as controls, and eight received one of three concentrations of the test substance. Communities colonized in the field and placed in the laboratory for the exposure generally contain the more resistant juvenile and adult life-stages. Following both types of exposure, the animals were collected by siphoning the contents of each aquarium into a 1-mm-mesh sieve, preserved and identified. Data were then analyzed and effects on community structure, including numbers of individuals, number of species,

species diversity indices and, occasionally effects on community function, were determined.

Results from eighteen community tests conducted over the last decade (Table 8) demonstrate that a variety of species of marine organisms will settle and grow in this system (Hansen, 1974; Hansen and Tagatz, 1980; Tagatz and Tobia, 1978; Tagatz and Ivey, 1981; Tagatz et al., 1977; 1978 a,b; 1979 a,b; 1981; 1982; In press[a] (unpublished data on carbophenothion and pentachlorophenol and di-n-butyl phthalate). A total of 54,000 individuals representing 223 species from 11 phyla have been identified from these 18 experiments. The numbers of individuals, species and phyla differed considerably among tests, probably as a function of the seasonal variations in reproductive patterns of species and water quality. The test-to-test differences were greatest in the number of individual organisms, 180 to 12,000 (average 3,000), but were less variable for the number of species per test, 24 to 81 (average 44); and phyla per test, 5 to 9 (average 7). Of the total of 223 species from the 18 tests, 45 percent were annelids, 23 percent mollusks, and 18 percent arthropods (Table 9). Species from the remaining 8 phyla constituted only 12% of the total. As a result, this test is generally most effective in detecting effects on species in the three dominant phyla.

A comparison of communities that developed in laboratory and field experiments (Table 8) shows that test-to-test variations are greatest in the number of individuals and more similar in the numbers of species and phyla. Tagatz and Deans (In press) discuss the degree of structural similarity of benthic communities in the field with those that develop in experimental aquaria in the field and laboratory and the relative merits of toxicity tests using laboratory- vs. field-developed communities. They concluded that, although each test has merit, the laboratory test is operationally simpler and

can be more sensitive because early life-stages of settling organisms are exposed.

An examination of data from the 18 community tests with pesticides, formulated products, organic chemicals, and muds used in drilling oil wells provides insight into the sensitivity of phylogenetic groups not possible in toxicity tests in which species are exposed individually (Table 10). As would be expected, arthropods were particularly sensitive to the five pesticides tested. Arthropods were not particularly sensitive to the other substances, except Dowicide G-ST which contains 79% sodium pentachlorophenate. Mollusca was the most frequently sensitive phylum. Its sensitivity to pesticides rivaled that of the arthropods and probably would not have been detected from acute mollusk larval toxicity tests or oyster shell deposition tests. Annelids, chordates (tunicates), echinoderms and coelenterates, phyla rarely tested as individual species in acute and chronic toxicity tests (Table 1 and 4), were also identified as particularly sensitive to certain organic chemicals and drilling muds. Surprisingly, decreases in numbers of individuals or species were compensated for by increases in other taxa only twice, with toxaphene and Aroclor 1254.

A comparison with results from single species tests demonstrates that community tests: (a) provide information on the sensitivity of species that cannot be determined from tests of species separately; (b) can be as sensitive as, or more sensitive than, chronic exposures of individual species because the often most sensitive early life-stages of a wide variety of species are exposed; (c) provide insight into the limits of applicability of single-species tests to predict impacts on communities consisting of many interacting species, some of which are phylogenetically related to species that are sensitive in single species tests. The test in which communities develop in the laboratory

as they are exposed is the most sensitive. However, caution must be used in extrapolation of the results from both of these community tests to environmental effects because test communities are not exact replicates of field communities, particularly in the absence of larger benthic and water column species and the variability of exposure conditions between the laboratory and the field.

UTILITY OF TOXICITY TESTS

The ultimate purpose of developing and using toxicity tests is to determine the toxicity of substances and the sensitivity of species, to use these data, along with data on environmental concentration, to predict with known accuracy the potential for environmental hazard, and to do so at minimum cost (Figure 2). To be cost effective, those substances with minimum or no likelihood for environmental harm must be rapidly identified with minimal expenditure of resources. It is essential that the majority of resources be available to study those substances that possess the greatest potential for hazard. Single- species toxicity tests, or structure-activity relationships derived from these tests and physical-chemical properties of classes of substances, are likely to be the most cost effective technique of identifying those substances that have a low degree of hazard.

Accurate predictions when hazard is likely to be high, i.e. where effect and environmental concentrations are similar, requires a maximum of resources and testing beyond that of single-species toxicity tests in which species and water differ from that of the specific site of concern (Figure 3). Even in such instances, testing will generally begin with standardized single-species toxicity tests that have the high degree of control and replicability needed to determine the toxicity of the substance, the species most sensitive, the stages of the life-cycle susceptible to sublethal exposure, the effect of site water

quality on toxicity and the bioavailability of the substance. These results are then used to select exposure concentrations and appropriate test design in the latter stages of the evaluation, where toxicity tests are conducted that measure effects on systems of interacting species, such as are found in microcosms, and in tests to measure impacts on community structure and function. Finally, in those instances where the substance is already in the marine environment, field studies may be conducted. Few field studies have attempted to provide data linking all aspects of this evaluative process. This is probably because of the expense and the fact that, except in engineered systems, the controls and replication so important in experimental design are lacking. Effective assessment of substances when the hazard is high requires integration of critical laboratory and field studies. The debate by environmental scientists can be most effective by emphasizing the overall process of hazard evaluation and how it can be more efficacious, rather than debating over who has the best test this week.

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Table 1. Frequency of occurence of acute toxicity test data on marine species of various phyla in the 64 consent-decree water quality criteria documents. Thirteen criteria documents contained no acute toxicity data on animals and 26 contained no phytoplankton or macroalgae data.

Phylum	Frequency of Testing of Species			Total Species
	1-5 Chemicals	6-10 Chemicals	>10 Chemicals	
Arthropoda	22	7	2	31
Chordata	17	5	4	26
Mollusca	8	2	1	11
Annelida	6	-	1	7
Echinodermata	1	-	-	1
Total Species	54	14	8	76
Phytoplankton	31	1	1	33
Macroalgae	7	1	-	8

Table 2. Variation in acute sensitivities of marine species to chlordane, DDT, dieldrin, endosulfan, endrin, heptachlor, cadmium, chromium, copper, mercury, nickel, selenium, silver, and zinc. Values are grand averages across substances, ranges for each substance in parentheses.

ITEM	PESTICIDES	METALS
Number of species tested	16 (8-21)	20 (10-26)
LC50 least sensitive/LC50 most sensitive	3,800 (70-18,000)	600 (20-2,300)
Sensitive phyla[a]	Arthropoda, Chordata	Arthropoda, Mollusca, Chordata, Annelida
Percentile Rank Of:		
Acartia spp.	33	28 (8-50)
Mysids	46 (29-67)	27 (3-70)
Penaeids	7 (5-12)	46 (38-61)

[a] Listed when a species from the phylum was one of the two most sensitive to the substances.

Table 3. Interlaboratory comparison of the results from static or flow-through acute toxicity tests. Results are mean 96-hour LC50's in µg/ℓ for six laboratories; coefficient of variation in parentheses (s/x) calculated from LC50's reported by Schimmel (1981). LC50's were calculated from nominal concentrations in exposure water in static tests and measured concentrations in flow-through tests.

SPECIES	CHEMICAL	STATIC	FLOW-THROUGH
C. variegatus	Endosulfan	2.40(0.37)	0.82(0.38)
	Silver	1,122(0.34)	1,216(0.46)
M. bahia	Endosulfan	0.80(0.62)	0.90(0.40)
	Silver	210(0.27)	192(0.58)
A. tonsa	Endosulfan	0.22(0.82)	-
	Silver	38.5(0.42)	-

Table 4. Species of marine fishes and invertebrates that have been tested in life-cycle or early life-stage toxicity tests and approximate number of tests completed.

Test	Species	Number of Tests
Partial or entire life-cycle	Cyprinodon variegatus	>10
	Mysidopsis bahia	>25
	M. bigelowi	2
	Palaemonetes pugio	2
	Acartia tonsa	1
	Crepidula fornicata	1
Early life-stage	Cyprinodon variegatus	>25
	Menidia menidia	2
	M. peninsulae	1
	Leuresthes tenius	1
	Opsanus beta	1
	Fundulus similis	1

Table 5. Summary of the chemicals where the MATC's from life-cycle toxicity tests were predicted within a factor of two by estimated MATC's from early life-stage portions of these life-cycle tests. The estimated MATC's used in these comparisons were from the progeny portions of partial life-cycle tests or from the progeny or parental portions of entire life-cycle tests.

Chemical	
MATC's Predicted	MATC's Not Predicted
Carbofuran, Chlordane Endrin, Heptachlor, Kepone, Malathion, Methoxychlor, Pentachlorophenol	Diazinon, EPN, Trifluralin

Table 6. Frequency of occurrence of survival, growth or reproduction (eggs/female or fertility) as endpoints in early life-stage or life-cycle toxicity tests with the fish (Cyprinodon variegatus), grass shrimp (Palaemonetes pugio) and bay mysid (Mysidopsis bahia).

TEST	NUMBER OF TESTS	MOST SENSITIVE ENDPOINT		
		SURVIVAL	GROWTH	REPRODUCTION
Early life-stage (Fish)	23	52%	52%	---
Life-cycle (Fish)	11	45%	27%	77%
Life-cycle (grass shrimp)	2	0%	0%	100%
Life Cycle (Bay mysid)	21	57%	19%	57%

Table 7. Application factors (no-effect concentration in a partial or entire life-cycle toxicity test divided by the 96-hour LC50) for the sheepshead minnow, an estuarine fish, vs. those of freshwater fishes tested against the same chemical.

Chemical	Application Factor	
	Sheepshead minnow	Freshwater fishes
Diazinon	<0.0003	0.03[a], <0.0004[b] <0.0007[c]
Endrin	0.35	0.26[a]
Heptachlor	0.09	0.12[b]
Kepone	0.001	0.004[b]
Malathion	0.08	0.02[b], 0.03[d], 0.06[a]
Trifluralin	0.007	0.017[b]

[a] *Jordanella floridae*

[b] *Pimephales promelas*

[c] *Salvelinus fontinalis*

[d] *Lepomis macrochirus*

Table 8. Number of individuals, species and phyla in eighteen laboratory- and field-colonized estuarine benthic community toxicity tests; range in parentheses.

	Individuals	Species	Phyla
Number per test (n=18)	3,000(180-12,000)	44(24-81)	7(5-9)
Average per laboratory-colonized test (n=14)	3,600(270-12,000)	42(24-81)	7(5-9)
Average per field-colonized test (n=4)	1,000(180-2,300)	50(32-65)	7(6-9)
Total all tests	54,000	223	11

Table 9. Number of species, by phylum, from eighteen laboratory- and field-colonized estuarine benthic community toxicity tests.

Phylum	Number of Species
Annelida	100
Mollusca	55
Arthropoda	40
Echinodermata	8
Chordata	5
Nemertinea	5
Coelenterata	4
Platyhelminthes	2
Sipunculoidea	2
Hemichordata	1
Phoronida	1
Total - 11 Phyla	223 Species

Table 10. Phyla whose numbers of individuals or species were reduced in estuarine benthic community toxicity tests with individual pesticides (chlorpyrifos, fenvalerate, pentachlorophenol, sevin or toxaphene); with formulated products (Dowicide-GST or Surflo-883); with non-pesticide organics (Aroclor 1254 or di-n-butyl phthalate) or insoluble energy-related products (barite, drilling muds incorporated in sand or muds delivered in suspension). Two tests were conducted with chlorpyrifos, fenvalerate and di-n-butyl phthalate and four tests with pentachlorophenol.

Phylum	Number of tests where phylum was reduced				
	Pesticides	Formulations	Organics	Muds	Total
Arthropoda	6	0	1	0	7
Mollusca	5(1[a])	2	2	0	9(1[a])
Annelida	1	0	1	2	4
Chordata	1	0	1(1[a])	1	3(1[a])
Echinodermata	0	0	1	0	1
Coelenterata	0	0	0	1	1
Total tests	10	2	3	3	18

[a] Numbers were increased in the presence of specific chemicals.

Figure Legends

Figure 1. - Diagram of the sheepshead minnow (*Cyprinodon variegatus*) entire life-cycle toxicity test.

Figure 2. - Optimization of the hazard evaluation process to minimize testing effort for substances with a low potential for hazard and to maximize effort for substances with the greatest potential hazard.

Figure 3. - Relationship between the degree of control, replication, cost and fidelity to the field of laboratory and field effects assessment metholologies.

ure 1

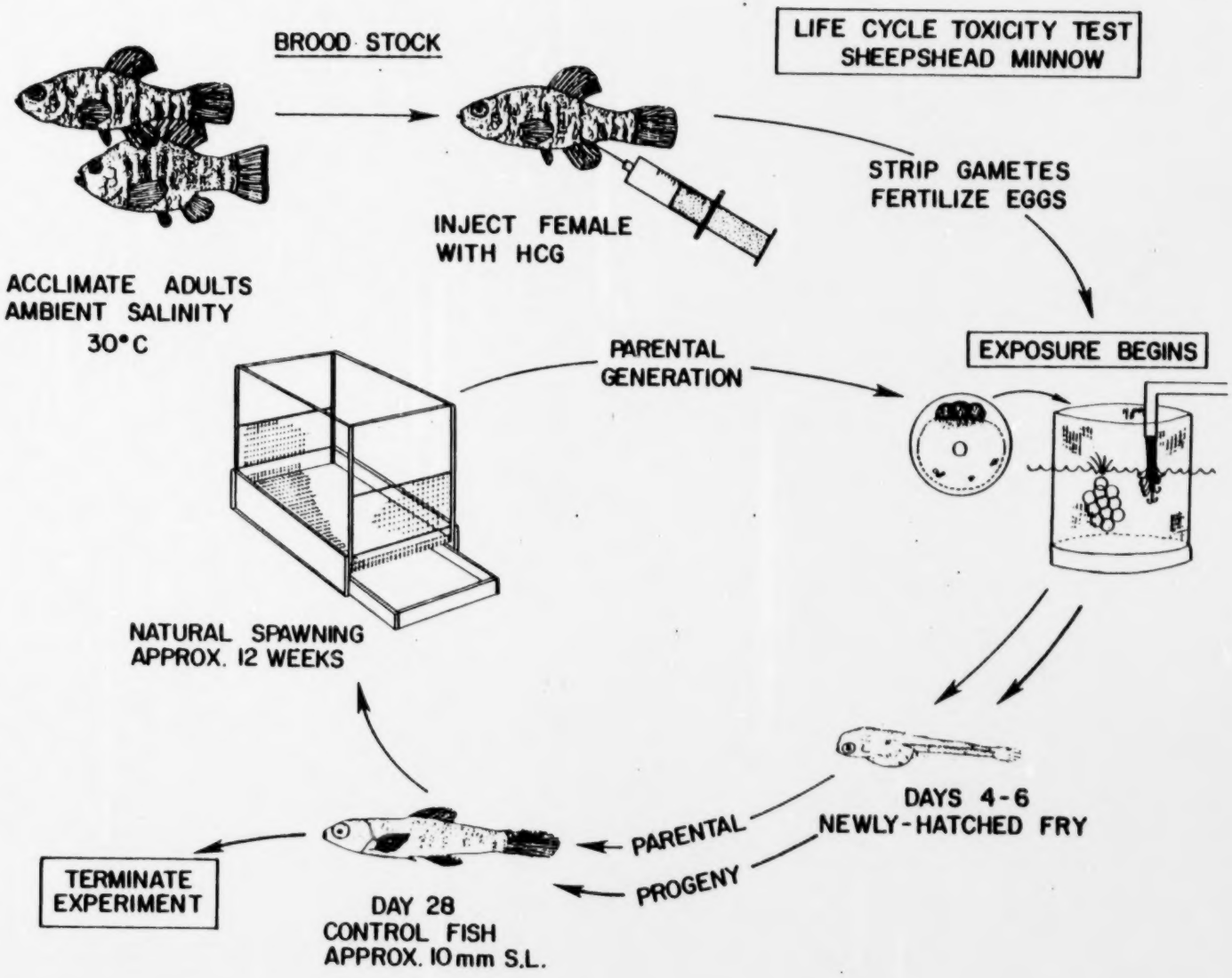

LIFE CYCLE TOXICITY TEST
SHEEPSHEAD MINNOW
BROOD STOCK
ACCLIMATE ADULTS
AMBIENT SALINITY
30°C
INJECT FEMALE
WITH HCG
STRIP GAMETES
FERTILIZE EGGS
EXPOSURE BEGINS
PARENTAL
GENERATION
NATURAL SPAWNING
APPROX. 12 WEEKS
DAYS 4-6
NEWLY-HATCHED FRY
PARENTAL
PROGENY
DAY 28
CONTROL FISH
APPROX. 10mm S.L.
TERMINATE
EXPERIMENT

Figure 2

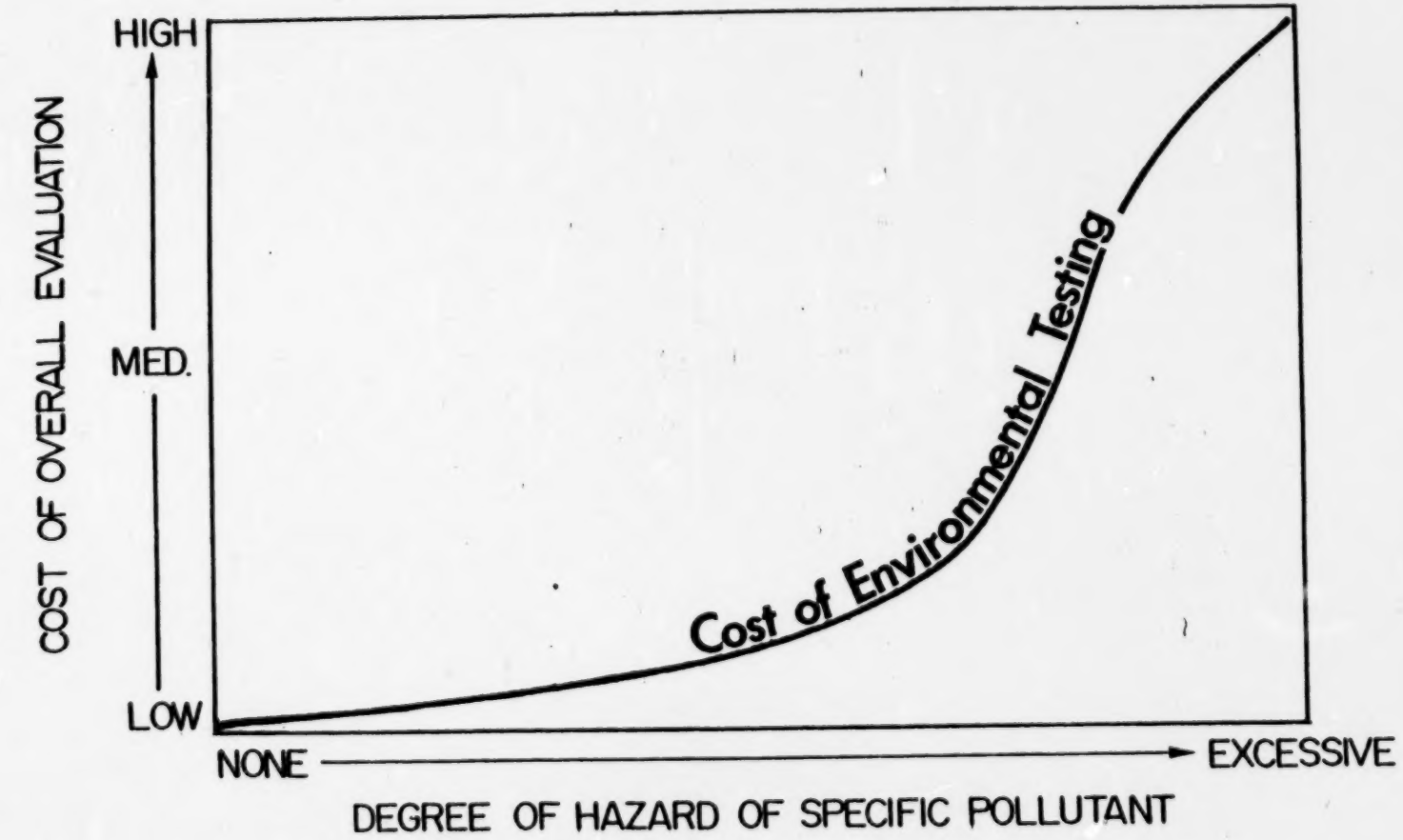
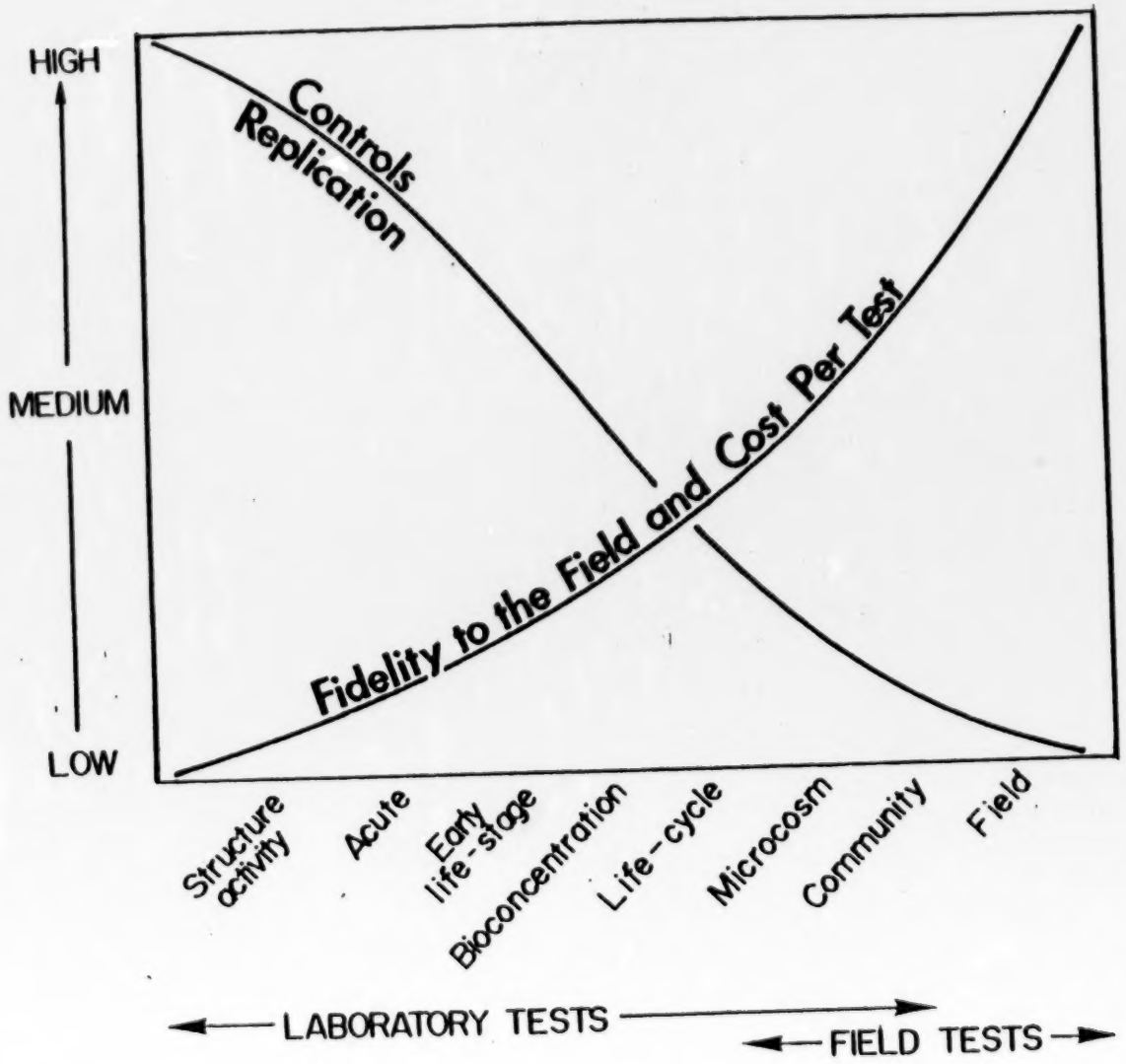

HIGH
MED.
LOW
COST OF OVERALL EVALUATION
Cost of Environmental Testing
NONE
EXCESSIVE
DEGREE OF HAZARD OF SPECIFIC POLLUTANT

Figure 3


HIGH
MEDIUM
LOW
Controls Replication
Fidelity to the Field and Cost Per Test
Structure activity
Acute
Early life-stage
Bioconcentration
Life-cycle
Microcosm
Community
Field
LABORATORY TESTS
FIELD TESTS